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Effects of 4-pyridine aldoxime on nerve agent-inhibited acetylcholinesterase activity in guinea pigs

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Abstract Methoxime (MMB-4) is a leading candidate oxime acetylcholinesterase (AChE) reactivator to replace pralidoxime (2-PAM) for therapeutic treatment of nerve agent intoxication. 4-Pyridine aldoxime (4-PA) is a synthetic starting material, a breakdown product, and a probable metabolite of MMB-4. There is a possibility that 4-PA may adversely interact with the nerve agent, thereby affecting nerve agent toxicity and biological AChE activity. This study evaluated the effects of 4-PA on sarin (GB)-, cyclosarin (GF)-, and VX-induced toxicity and AChE activity in blood, brain, and peripheral tissues of guinea pigs. Animals were pretreated with atropine methyl nitrate (1.0 mg/kg, im) 15 min prior to subcutaneous administration with 1.0× LD_{50} of GB, GF, or VX and then treated 15 min after the administration of nerve agents with 4-PA (3.5, 7.0, or 14.0 mg/kg, im). The dose–response effects of 4-PA alone were also examined. Toxic signs and lethality were monitored, blood and tissues were collected, and AChE activities were determined at 60 min after nerve agent administration. Under the condition of this study, all animals exposed to nerve agents exhibited some degree of toxic signs such as salivation, lacrimation, rhinorrhea, and convulsions. 4-PA at the three doses tested neither induced toxic signs nor altered the toxicity of GB, GF, or VX at the 1.0× LD_{50} exposure dose. Additionally, it did not modify the AChE activity in blood, brain, and peripheral tissues by itself or affect the AChE activity inhibited by a 1.0× LD_{50} dose of these three nerve agents in guinea pigs.

Keywords Acetylcholinesterase · Cholinesterase · Cyclosarin · Guinea pig · Methoxime · Organophosphorus compounds · Oximes · Pralidoxime · 4-Pyridine aldoxime · Sarin · VX

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the *Guide for the Care and Use of Laboratory Animals*, by the Institute of Laboratory Animal Resources, National Research Council. The research environment and protocols for animal experimentation were approved by the Institutional Animal Care and Use Committee (IACUC) of the US Army Medical Research Institute of Chemical Defense. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
AMN	Atropine methyl nitrate
ChE	Cholinesterase
BCA	Bicinchoninic acid
DTNB	5,5′-Dithiobis-2-nitrobenzoic acid
GB	Sarin
GF	Cyclosarin
im	Intramuscular
LD_{50}	Median lethal dose
MMB-4	Methoxime
OP	Organophosphorus compound
2-PAM	Pralidoxime; pyridine-2-aldoxime methylchloride
4-PA	4-Pyridine aldoxime
PB	Pyridostigmine bromide
RBC	Red blood cell

sc Subcutaneous
WB Whole blood

Introduction

Organophosphorus (OP) nerve agents, such as sarin (GB), cyclosarin (GF), and VX, are potent cholinesterase (ChE) inhibitors. It has generally been recognized that the acute toxic manifestations of exposure to OP nerve agents are due to their irreversible binding to the ChE class of enzymes, in particular acetylcholinesterase (AChE), which serves to hydrolyze and degrade the released cholinergic neurotransmitter acetylcholine (ACh) at the synaptic junction of the central and peripheral cholinergic nervous systems and the neuromuscular junction (Taylor 2001). Excess ACh results in uncontrolled stimulation followed by blockade of neuronal transmission. Reactivation of inhibited AChE is identified as a reasonable pharmacologic approach, leading to the use of 2-PAM (pralidoxime; pyridine-2-aldoxime methylchloride) and obidoxime (Toxogonin®) for treatment of OP poisoning over 50 years ago (Wilson and Ginsburg 1955; Childs et al. 1955; Hobinger and Sadler 1959). These oxime compounds react with the AChE-OP complex to displace the phosphoryl group and restore normal enzymatic activity. They also increase the efficacy of atropine sulfate, which serves to counteract the buildup of excess ACh at cholinergic synapses throughout the body.

Even though several oxime reactivators such as 2-PAM, P2S (N-methylpyridinium-2-aldoxime methanesulfonate), obidoxime (Toxogonin®), TMB-4 (trimethoxime), MMB-4 (methoxime), or HI-6 (1-(4-carbamoylpyridino) methoxymethyl-2-(hydroxyiminomethyl) pyridinium) have been clinically used for therapy of OP poisoning in many countries around the world, the only licensed oxime in the US for the treatment of nerve agent exposure is 2-PAM (Moore et al. 1995; Aas 2003). While 2-PAM has acceptable efficacy against certain nerve agents (e.g., GB, VX), it lacks the desired level of efficacy against other nerve agents (e.g., tabun, soman, GF), even when combined with pyridostigmine bromide (PB) pretreatment and atropine and diazepam treatment (Boskovic et al. 1984). Additionally, the safety index (the ratio of lethal dose to effective dose) of 2-PAM is relatively low (4.4–8.4). This limited and narrow efficacy against only certain OP nerve agents represents an unmet need for effective medical pharmacological management of nerve agent casualties in chemical warfare theater for military operations. Thus, research to identify and develop broad spectrum AChE reactivators becomes a necessity.

MMB-4 is currently being proposed as a leading candidate for replacement of 2-PAM as an oxime antidote to reactivate nerve agent-inhibited AChE activity and alleviate toxic and lethal consequences that a nerve agent exposure can cause (Singh et al. 2007; Saxena et al. 2008). The chemical compound 4-pyridine aldoxime (4-PA) is a synthetic starting material, a breakdown product, and a probable metabolite of MMB-4 (Fig. 1). There is also a trace amount of 4-PA in the MMB-4 formulation. The interaction of 4-PA with AChE in vivo may complicate the pharmacological consequence of nerve agent therapy. Therefore, the purposes of this study were to determine the capacity of 4-PA to affect the toxicity of the nerve agents and/or to interact with AChE activity (either inhibit or reactivate) in peripheral tissues and the central nervous system (CNS) after nerve agent (GB, GF, or VX) intoxication in guinea pigs.

Materials and methods

Atropine methyl nitrate (AMN), 4-pyridine aldoxime (4-PA), bovine serum albumin, Triton-X100, and acetylthiocholine iodide were purchased from Sigma–Aldrich (St. Louis, MO). Saline (USP) was purchased from Braun Medical Inc. (Irvine, CA). Heparin sodium was purchased from USP, Inc. (Rockville, MD). DTNB (5,5′-dithiobis(2-nitrobenzoic acid), bicinchoninic acid (BCA) Protein Assay Reagent A (sodium carbonate, sodium bicarbonate, BCA™ detection reagent, and sodium tartrate in 0.1 N sodium hydroxide) and BCA Protein Assay Reagent B (4% cupric sulfate) were purchased from Pierce Biotechnology, Inc. (Rockford, Illinois). DTNB was prepared in Tris buffer (0.05 M, pH 8.2) to a concentration of 0.424 M. The nerve

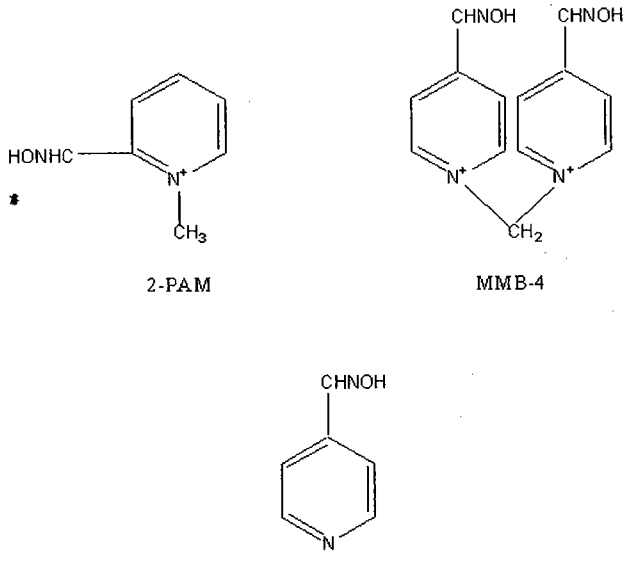


Fig. 1 Chemical Structures of 2-PAM, MMB-4, and 4-PA

agents, sarin (GB), cyclosarin (GF), and VX were obtained from the US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. Injection solutions were prepared in normal saline on the day of the experiment in a volume of 0.5 ml/kg. GB, GF, and VX were injected subcutaneously (sc), whereas 4-PA and atropine methyl nitrate were administrated intramuscularly (im).

Male Hartley guinea pigs (Crl:(HA) BR COBS) weighing 250–350 g were purchased from Charles River Labs (Kingston, NY). They were housed in individual cages in temperature (21 ± 2°C)- and humidity (50 ± 10%)-controlled quarters that were maintained on a 12-h light–dark schedule (with lights on at 0600 hours). Laboratory chow and tap water were freely available whenever the animals were in home cages. Animals were allowed to acclimate for one week prior to experimentation.

Blood (0.25–0.5 ml) was drawn using the toenail clip method (Vallejo-Freire 1951) and collected into a 1-ml microfuge tube containing 50 µl of heparin sodium (15 units/ml in saline) 1 to 3 days prior to the experimentation to establish baseline red blood cells (RBCs) and whole blood (WB) AChE activity.

On the day of the study, the guinea pigs were pretreated with atropine methyl nitrate (AMN; 1.0 mg/kg, im) 15 min prior to a nerve agent exposure to minimize peripheral toxic effects. AMN is a peripheral acting muscarinic receptor blocker that does not affect AChE activity. Animals were challenged with a 1× LD_{50} subcutaneous dose of GB, GF, or VX. 4-PA was given im 15 min later, at the time of maximum brain, blood, and tissue AChE inhibition by the 1.0× LD_{50} dose of a nerve agent (Shih et al. 2005). A saline/saline control group, a nerve agent-exposed/saline-treated group, and three 4-PA groups (3.5, 7.0, or 14.0 mg/kg) were tested following a sc 1.0× LD_{50} nerve agent challenge. Also, the dose–response effects of 4-PA alone were studied.

These three doses (3.5, 7.0, or 14.0 mg/kg) of 4-PA were tested based on the following considerations. Like HI-6, MMB-4 is a bispyridinium compound. The maximum three autoinjector doses of HI-6 to be given to a 70-kg person is 58 µmol/kg, im (Clair et al. 2000; Aas 2003), so the maximum dose of MMB-4 dimethanesulfonate to be given will be ~28 mg/kg, im, per person (Singh et al. 2007). Therefore, MMB-4 degraded by 12.5, 25, and 50% should result in 3.5, 7.0, and 14 mg/kg, respectively, of 4-PA metabolite in the body. Based on the blood volume of a normal guinea pig (Ancill 1956), these doses of 4-PA should cause a maximum blood concentration of 89.5, 179, and 358 µg/ml, respectively, of 4-PA in the circulation plasma of a guinea pig, which may be physiologically significant enough to induce pharmacological or toxicological consequences.

Cholinergic toxic signs were scored at ~13 min (before 4-PA administration) and ~58 min (before termination of the experiment) after a 1.0× LD_{50} nerve agent injection. Toxic signs were rated using the following scores: general motor signs (0 = normal, 1 = fasciculation, 2 = tremor, and 3 = convulsions); general state (0 = normal coordination, 1 = mildly uncoordinated, 2 = impaired movement, and 3 = prostrated); nystagmus (0 = absent and 1 = present); lacrimation (0 = absent and 1 = present); and salivation (0 = absent and 1 = present). A total toxic sign score was calculated based on the sum of each animal's highest observed score from each of the five criteria (see Table 1).

Sixty min after GB, GF, or VX administration, the animals were deeply anesthetized with isoflurane and euthanized by decapitation. Blood (0.25–0.5 ml) was collected into a 1.0-ml microfuge tube containing 50 µl of heparin sodium solution (15 U/ml). For the WB samples, 20 µl of blood was diluted 1:25 in 1% Triton–X100 solution. For the RBC samples, the original blood sample was centrifuged for 5 min at 16,000×g, and 10 µl of the RBC was then diluted 1:50 in 1% Triton–X100 solution. Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, and striatum) and peripheral tissue (diaphragm, heart, and skeletal muscle) were dissected. Brain samples were diluted 1:20, while peripheral samples were

Table 1 The cholinergic toxicity sign scoring system

Score given per condition[a]				
	0	1	2	3
General motor signs	Normal	Fasciculation	Tremor	Convulsions
General state	Normal	Mildly uncoordinated	Impaired movement	Prostrated
Salivation	Absent	Present		
Lacrimation	Absent	Present		
Nystagmus	Absent	Present		

[a] Each guinea pig was scored on each of the five categories of cholinergic signs induced by organophosphorus nerve agents. The scores for general motor signs and general state were 0 (normal), 1 (mild), 2 (moderate), or 3 (severe), whereas the scores for salivation, lacrimation, and nystagmus were based on absence (0) or presence (1) of symptoms. The total scores for each animal were then summed from the maximum score from each of the five categories. The maximum severity score was 9 for each animal

diluted 1:5, in 1% Triton-X100 solution (in water) and then homogenized. The homogenates were then centrifuged (31,000×*g* at 4°C; 20 min for brain and 30 min for peripheral tissues), and the supernatant was decanted and kept frozen at −80°C until analysis. In all these experiments, collected blood, brain, and peripheral tissue samples were analyzed for AChE activity using a variation of the microplate method modified from Ellman et al. (1961), reported elsewhere (Shih et al. 2005).

Protein levels in the brain and peripheral tissue samples were determined by a BCA protein assay method (Pierce Biotechnology, Inc.). The standard curve was created using bovine serum albumin at the following concentrations: 0.5, 0.75, 1.0, 1.5, and 2.0 mg/ml. Three replicates of 10 µl for each brain sample were added to individual microplate wells. To each well of brain samples, 200 µl of working reagent was then added. Three replicates of 5 µl for each peripheral tissue sample were added to individual microplate wells. The peripheral tissue samples were further diluted by adding 5 µl of deionized water before adding 200 µl of BCA working reagent. The microplates were then incubated at 37°C for 30 min. The microplates were allowed to cool to room temperature (15 min) before being read using a Spectramax Plus 384 microplate reader and Softmax Plus 4.3 LS software (Molecular Devices, Sunnyvale, CA). A single measurement of absorbance was made at 562 nm, and protein concentrations were extrapolated from the standard curve.

AChE activity was expressed initially as µmol/ml/min for RBC and WB samples and as µmol/g protein/min for brain and peripheral tissue samples. The enzymatic activities of the treatment groups were then expressed as percentages of the saline/saline control group (mean ± SEM % of control value) for each nerve agent. A one-way analysis of variance (ANOVA) was used to compare AChE activity across treatment groups with respect to each tissue group. A Kruskal–Wallis test was used to compare treatment groups with respect to toxic sign scores. Additionally, a Wilcoxon Signed Rank test was used to compare toxic signs before and after treatment for each dose of 4-PA or saline. Statistical significance for all statistical tests was defined as $P \leq 0.05$.

Results and conclusion

Effects of 4-PA on nerve agent-induced toxic signs

As shown in Table 2a and b, 13 min following 1.0× LD_{50} GB or GF administration, guinea pigs exhibited toxic signs of classical cholinergic crisis. These include muscle fasciculations, tremors, convulsions/seizures, loss of motor coordination, prostration, nystagmus, lacrimation, and salivation. These toxic signs progressed and became more severe at 58 min. The toxic scores resulted from GB and GF exposure increased from 3.00–3.75 at 13 min (before 4-PA administration) to 5.00–7.00 at 58 min (before termination of the experiment). Similarly, during this same period of time, the toxic sign scores for the 4-PA-treated groups increased by approximately 1.10–2.25 points. VX-challenged groups (Table 2c) displayed a delay in the onset of toxic signs compared to those challenged with GB or GF in that few signs were apparent prior to 4-PA treatment at 13 min. At 58 min, the toxic sign scores of the 4-PA-treated groups showed no statistical difference from those induced by GB, GF, or VX alone. Thus, 4-PA did not modify the severity of the nerve agent-induced toxic sign score. There were no toxic signs induced by any dose of 4-PA alone (data not shown).

Table 2 Effects of 4-PA on toxic signs following GB, GF, or VX intoxication

Treatment (mg/kg)	Before oxime (~13 min)	~58 min	*N*
a			
Saline/saline	0.00	0.00	6
Saline/4-PA (14)	0.00	0.00	2
GB/saline	3.00 (±0.55)	5.00 (±1.14)	5
GB/4-PA (3.5)	3.38 (±0.87)	5.38 (±1.17)	8
GB/4-PA (7)	4.50 (±0.38)	6.25 (±0.75)	8
GB/4-PA (14)	4.25 (±0.75)	6.50 (±0.38)	8
b			
Saline/saline	0.00	0.00	6
Saline/4-PA (14)	0.00	0.00	2
GF/saline	3.75 (±1.03)	7.00 (±0.41)	4
GF/4-PA (3.5)	4.22 (±0.52)	5.33 (±0.82)	9
GF/4-PA (7)	4.44 (±0.53)	6.22 (±0.32)	9
GF/4-PA (14)	3.90 (±0.74)	5.00 (±0.88)	10
c			
Saline/saline	0.00	0.00	6
Saline/4-PA (14)	0.00	0.00	2
VX/saline	0.00	2.00 (±2.00)	3
VX/4-PA (3.5)	0.00	2.75 (±0.96)	8
VX/4-PA (7)	0.25 (±0.25)	2.13 (±0.83)	8
VX/4-PA (14)	0.00	3.30 (±0.93)	8

Guinea pigs were treated with atropine methyl nitrate (1.0 mg/kg, im) 15 min prior to challenge with GB, GF, or VX (1.0× LD_{50}, sc). 4-PA (3.5, 7.0, or 14.0 mg/kg, im) was given 15 min (at time of maximum brain, blood, and tissue AChE inhibition) after GB, GF, or VX challenge. Toxic signs were scored at ~13 min (before 4-PA administration) and at ~58 min (before termination of the experiment) following agent exposure and were expressed as mean ± SEM. No statistical difference in toxic sign scores was found between nerve agent/saline and any 4-PA-treated group

Effects of 4-PA on AChE activity inhibited by nerve agents

At 60 min after 1.0× LD_{50} GB, GF, or VX exposure, the AChE activity decreased below 25% of control levels in RBC and WB (Fig. 2). Treatments with any of the three tested doses (3.5, 7.0 or 14.0 mg/kg, im) of 4-PA at 15 min post-intoxication did not affect blood AChE activity inhibited by any nerve agent challenge. Figure 3 shows the AChE activity in brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, diaphragm, heart, and skeletal muscle following GB, GF, VX, or GB plus 4-PA, GF plus 4-PA, or VX plus 4-PA treatments. As is shown, each agent (at 1.0× LD_{50}) produced a different degree of AChE inhibition in different brain regions and peripheral tissues. However, the AChE activity observed at 60 min following any nerve agent exposure was not significantly affected by any of the doses of 4-PA administered at 15 min following GB, GF, or VX challenge.

Effects of 4-PA alone on AChE activity

4-PA at doses of 3.5, 7.0, or 14.0 mg/kg, im, did not alter the AChE activity in blood, brain regions, or peripheral tissues of guinea pigs (Fig. 4).

Summary

4-PA is a synthetic starting material, a breakdown product, and a probable metabolite of MMB-4. Because MMB-4 is a potential replacement for 2-PAM for therapy of OP nerve agent exposure (Singh et al. 2007; Saxena et al. 2008), we investigated the effects of 4-PA on blood and tissue AChE in vivo and its gross toxicity with concentrations (representing up to 50% metabolite of MMB-4) that may be reached in blood or tissues of the guinea pig. 4-PA is also an oxime by its own right with the oxime group at position 4 of the pyridine ring (Figure 1). Therefore, the capacity of 4-PA to affect toxicity and/or to interact with AChE (either inhibit or reactivate) in peripheral tissues and the central nervous system (CNS) after nerve agent (GB, GF, or VX) intoxication in guinea pigs was investigated. The interaction of 4-PA with AChE in vivo, if any, may complicate the pharmacological and toxicological consequences of nerve agent therapy.

Although, some oximes, such as 2-PAM, have been shown to inhibit ChE activity at concentrations of the order of 0.01 mM (Childs et al. 1955; Holmes and Robins 1955), 4-PA at the highest dose (14 mg/kg, im) used in the present study neither affected the AChE activity in blood, brain, or peripheral tissues nor modified the AChE activity inhibited by GB, GF, or VX. In the latter case, it was interesting to note that 4-PA, unlike 2-PAM, is a tertiary oxime without a charge. This structural arrangement rendered it lacking in

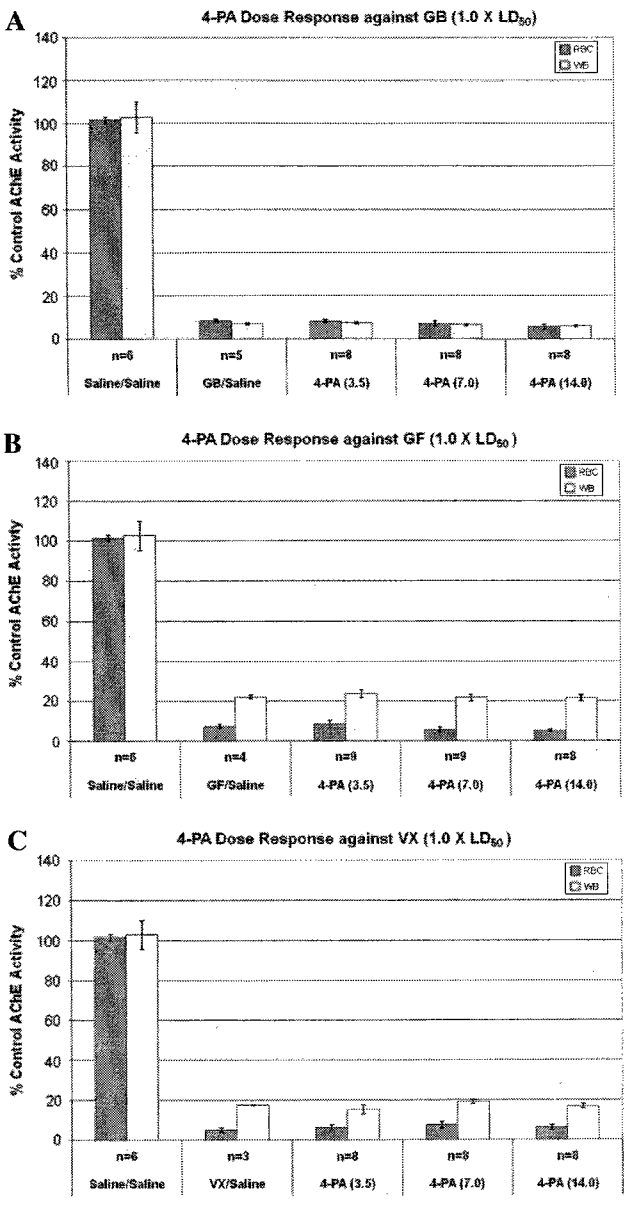
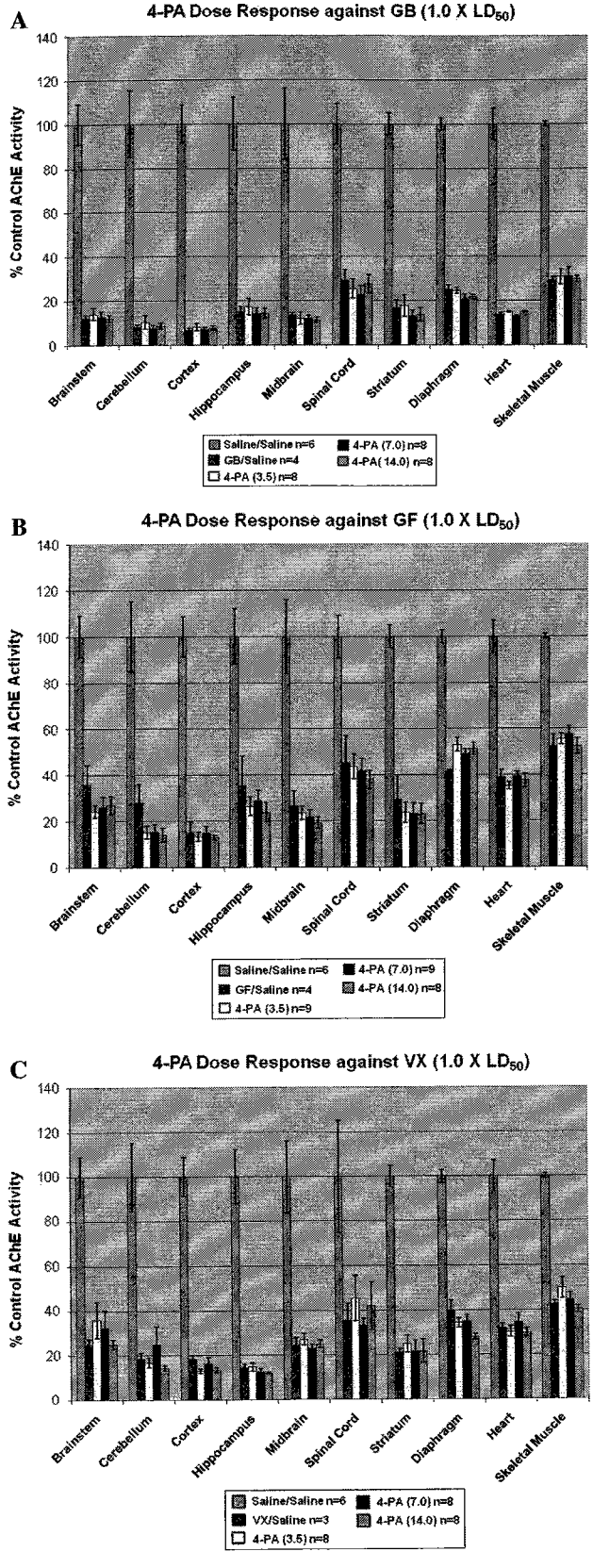


Fig. 2 The dose–response effects of 4-PA on GB (2A)-, GF (2B)-, or VX (2C)-inhibited acetylcholinesterase (AChE) activity in the red blood cell (RBC; *shaded bar*) and whole blood (WB; *blank bar*) of the guinea pig. Animals were treated with atropine methyl nitrate (1.0 mg/kg, im) 15 min prior to challenge with GB, GF, or VX (1.0× LD_{50}, sc). 4-PA (3.5, 7.0, or 14.0 mg/kg) was given im at 15 min (at time of maximum brain, blood, and tissue AChE inhibition) after agent challenge. Blood samples were taken at 60 min after nerve agent challenge. Data are expressed as percent of saline/saline control AChE activity with mean ± SEM. No statistical difference was found among test groups

nucleophilic power to reactivate AChE by cleavage of phosphonylated active sites, even though 4-PA may penetrate the CNS due to its tertiary structure. Another interesting observation was that at the higher dose studied, 4-PA, unlike 2-PAM, did not interact with the Ellman AChE assay substrate acetylthiocholine (Sakurada et al. 2006), in



◀ **Fig. 3** The dose–response effects of 4-PA on GB (3A)-, GF (3B)-, or VX (3C)-inhibited acetylcholinesterase (AChE) activity in brain regions and peripheral tissues of the guinea pig. Animals were treated with atropine methyl nitrate (1.0 mg/kg, im) 15 min prior to challenge with GB, GF, or VX (1.0× LD_{50}, sc). 4-PA (3.5, 7.0, or 14.0 mg/kg) was given im at 15 min (at time of maximum brain, blood, and tissue AChE inhibition) after agent challenge. Brain and peripheral tissues were harvested at 60 min after nerve agent challenge. Data are expressed as percent of saline/saline control AChE activity with mean ± SEM. No statistical difference was found among test groups

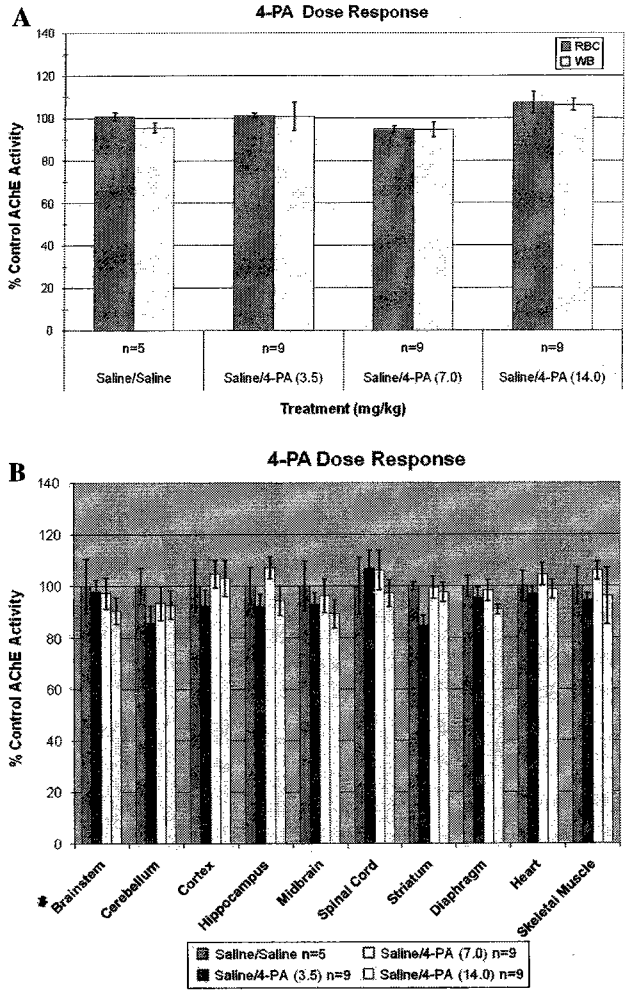


Fig. 4 The dose–response effects of 4-PA on acetylcholinesterase (AChE) activity in **a** the red blood cell (RBC; *shaded bar*) and whole blood (WB; *blank bar*), and **b** brain regions and peripheral tissues of the guinea pig. Animals were treated with atropine methyl nitrate (1.0 mg/kg, im) 30 min prior to 4-PA (3.5, 7.0, or 14.0 mg/kg, im) injection. Blood samples and tissues were taken at 60 min after saline administration. No nerve agent was administered. Data are expressed as percent of saline/saline control AChE activity with mean ± SEM. No statistical difference was found among test groups

particular in the blood samples where the higher concentration of 4-PA (i.e., 358 μg/ml plasma) is expected.

The present findings indicated that 4-PA alone at the three doses (3.5, 7.0, or 14.0 mg/kg, im) tested in this study did not induce toxic signs or change the AChE activity in blood, brain regions, or peripheral tissues of the guinea pig. Additionally, it neither altered the toxicity of GB, GF, or VX at a 1.0× LD_{50} exposure dose nor modified the AChE activity in blood, brain, or peripheral tissues inhibited by a 1.0× LD_{50} dose of these three nerve agents in guinea pigs. Thus, the existence of a trace amount of 4-PA may not have adverse systemic consequences complicating the use of MMB-4 in OP nerve agent therapy.

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References

Aas P (2003) Future considerations for the medical management of nerve-agent intoxication. Prehosp Disaster Med 18:208–216

Ancill RJ (1956) The blood volume of the normal guinea pig. J Physiol 132:469–475

Boskovic B, Kovacervic V, Jovaniovic D (1984) PAM-2 Cl, HI-6, and HGG-12 in soman and tabun poisoning. Fundam Appl Toxicol 4:S106–S115

Childs AF, Davies DR, Green AL, Rutland JP (1955) The reactivation by oximes and hydroxamic acids of cholinesterase inhibited by organophosphorus compounds. Br J Pharmacol Chemother 10:462–465

Clair P, Wiberg K, Granelli I, Carlsson BI, Blanchet G (2000) Stability study of a new antidote drug combination (Atropine-HI-6-prodiazepam) for treatment of organophosphate poisoning. Euro J Pharmaceut Sci 9:259–263

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Hobinger F, Sadler PW (1959) Protection against lethal organophosphate poisoning by quaternary pyridine aldoximes. Br J Pharmacol Chemother 14:192–201

Holmes R, Robins EL (1955) The reversal by oximes of neuromuscular block produced by anticholinesterases. Br J Pharmacol Chemother 10:490–495

Moore DH, Clifford CB, Crawford IT, Cole GM, Baggett JM (1995) Review of nerve agent inhibitors and reactivators of acetylcholinesterase. In: Quinn DM, Balasubramanian AS, Doctor BP, Taylor P (eds) Enzymes of the cholinesterase family. Plenum Press, New York, pp 297–304

Sakurada K, Ikegaya H, Ohta H, Akutsu T, Takatori T (2006) Hydrolysis of an acetylthiocholine by pralidoxime iodide (2-PAM). Toxicol Lett 166:255–260

Saxena A, Luo C, Chilukuri N, Maxwell DM, Doctor BP (2008) Novel approaches to medical protection against chemical warfare nerve agents. In: Romano JA, Lukey JA, Salem H (eds) Chemical warfare agents: chemistry, toxicology and therapeutics, 2nd edn. Pharmacology, CRC Press, Boca Raton, pp 145–173

Shih T-M, Kan RK, McDonough JH (2005) In vivo cholinesterase inhibitory specificity of organophosphorus nerve agents. Chem Biol Interact 157–158:293–303

Singh H, Moorad-Doctor D, Ratcliffe RH, Wachtel K, Castillo A, Garcia GE (2007) A rapid cation-exchange HPLC method for detection and quantification of pyridinium oximes in plasma and tissue. J Analy Toxicol 31:69–74

Taylor P (2001) Anticholinesterase agents. In: Hardman JG, Limbird LE, Gilman AG (eds) Goodman and Gilman's the pharmacological basis of therapeutics, 10th edn. McGraw-Hill, New York, pp 175–191

Vallejo-Freire AA (1951) A simple technique for repeated collection of blood samples from guinea pigs. Science 114:524–525

Wilson IB, Ginsburg S (1955) Reactivation of acetylcholinesterase inhibited by alkylphosphonates. Arch Biochem Biophys 54:569–571